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Research Article

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Development and Characterization of Green Tea Functional Beverages Infused with Black Ginger (*Kaempferia parviflora*)

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Abstract

Black ginger (*Kaempferia parviflora*) is a traditional herb from Southeast Asia packed with polymethoxyflavones (PMFs) which are known to have antioxidant, anti-fatigue and anti-ageing effects. Therefore, a functional drink was developed by combining black ginger and green tea to enhance its nutritional and antioxidant values. The absorbance of total phenolic content (TPC) has found to be increasing with increased extract concentrations. The TPC increased with increasing extract concentration, reaching 506.0 ± 1.7, 553.0 ± 1.6, and 573.3 ± 1.6 µg gallic acid equivalents per milliliter (µg GAE/mL) at extract concentrations of 10, 20, and 30 µg/mL, respectively. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity also increased with time and reached 88.70 ± 0.6% after 4 min. Proximate analysis indicated a moisture content of 1.289 ± 0.05%, crude fiber at 19 ± 0.9%, fat content at 0.62 ± 0.01%, and ash content of 9.49 ± 0.07%. The titratable acidity was 0.263 ± 0.01% and the yield from aqueous extraction was 21.34 ± 0.3%. The sensory evaluation was also carried out for three different treatments, i.e., T1 (control) and sample T2, T3, and T4, in which T2 sample was found most favorable with respect to taste, aroma and overall preference. These findings indicate that the fortification of black ginger to green tea provides promising antioxidant, nutritional and sensory benefits and can be developed as a functional beverage.

Keywords

Green tea, Functional beverage, Black ginger, Fortification, Antioxidant, Nutritive value

Introduction

Green tea has become popular recently due to its possible health benefits, such as neuroprotective, anti-inflammatory and antioxidant properties. It is gaining popularity among researchers as well as the general public due to the increasing interest in natural health treatments and lifestyle wellness. It is mostly made from *Camellia sinensis* leaves. Its history goes back to ancient China where it was used in traditional medicine and as a popular drink. Unlike oolong and black tea, green tea is processed to prevent the leaf from oxidizing. This minor processing method is likely to preserve catechins and natural polyphenols which are considered to have important health promoting properties [1]. Epigallocatechin-3-gallate (EGCG) is one of the catechins and has attracted the most attention because of its active physiological actions. These compounds have been linked to a lower risk of developing chronic diseases such as heart disease, many types of cancer, and neurological disorders according to Pervin et al. [2]. The ritualistic aspect of this is seen in the importance of tea in social and spiritual life and in the importance of tea's purported cleansing properties [3]. Green tea has been used as medicine for hundreds of years and is a popular beverage today that balances physiological

systems according to traditional Chinese medicine.

Several clinical and epidemiological studies have suggested that drinking green tea daily can improve overall health. For instance, Zhou et al. [4] reported that Japanese adults who drank more green tea were less likely to die from cardiovascular diseases. Moreover, the polyphenols in green tea have been shown to reduce inflammation and oxidative stress, two of the key factors involved in the development of chronic diseases [5]. Green tea has also been the focus of research into the treatment of obesity and type 2 diabetes due to its potential in improving insulin sensitivity and fat oxidation [6]. Adenosine monophosphate-activated protein kinase (AMP-AMPK), a master regulator of energy homeostasis, is believed to regulate these activities through its interactions with specific metabolic enzymes and signaling pathways. Several studies have also found that green tea can help improve cognitive abilities and postpone cognitive decline in older individuals [7]. Though further long-term clinical studies are required to understand effects of green tea on cognitive abilities.

Black ginger has been used in traditional Thai medicine to treat various illnesses such as sexual dysfunction, fatigue, allergies, and gastrointestinal problems [8]. It has been a great focus of scientific research for the past decade with accumulating proof of its adaptogenic and ergogenic capacities. The plant and rhizome are shown in figure 1a and 1b for easy identification. The extracts of green tea have also shown to improve physical performance, enhance mitochondrial function, and reduce the risk of metabolic diseases such as obesity and diabetes in clinical and preclinical studies [9]. The herb is believed to work by activating AMP-AMPK, nitric oxide production and regulation of inflammatory pathways [10]. Flavonoids are a major phytochemical constituent in black ginger, particularly PMFs like 5,7-dimethoxyflavone have shown to have potent biological effects which make it a potent antioxidant while lowering oxidative stress and cellular damage [11]. Similarly, black ginger has also shown to have antimicrobial, anti-cancer, and neuroprotective properties [12]. This has created interest in applying it as a natural dietary supplement in metabolic health products, sports nutrition and anti-ageing treatment. Research has also indicated that black ginger extract can boost endurance by supporting mitochondrial function in skeletal muscle, regulating energy metabolism, and increasing glycogen utilization. Research also suggests that supplementation can improve muscle strength in young athletes as well as reduce stress levels [13]. Thus, infusing green tea with black ginger may contribute to overall health improvement. While green tea enhances mental clarity and provides antioxidants, black ginger supports metabolic health and physical performance. They cater to the growing consumer interest in natural products that promote both mental and physical well-being. Owing to their combined health benefits and compatibility with the current consumer trend favoring natural and functional items, green tea (*C. sinensis*) and black ginger (*K. parviflora*) were chosen as key ingredients in product development. A lesser-known member of the ginger family, black ginger *(K. parviflora),* has long been used in Thai medicine due to its numerous medicinal uses, making this research effort especially significant. While it has been utilized for centuries in Southeast Asia, it has only recently started to attract scientific interest outside of its indigenous area. This categorizes it as a significant but insufficiently examined resource in the areas of functional foods and herbal remedies.



Figure 1: Black ginger **(a)** plant and **(b)** rhizome.

Materials and Methods

Materials

All chemicals used were purchased from Sisco Research Laboratories Pvt. Ltd., India, and HiMedia Laboratories Pvt. Ltd., India. The instruments and tools used are an ultrasonic cleaner (LMUC-12, Labman Scientific Instruments Pvt Ltd., India), a centrifuge (R-8C plus, Remi Eleketrotechnik Ltd.), a refrigerator (RT EONALOR 310/2024), a ultraviolet-visible (UV-Vis) spectrophotometer (Thermo-Scientific, Singapore), a muffle furnace (NAVYUG, India), a rotary flask shaker (Model LT-5156), a heating mantle (Remi), a hot air oven (HTLP 105 L), a Soxhlet apparatus (Modern Industry Kolkata), and a tray dryer (R-G-100-50).

Sample collection

The green tea leaves were obtained from a local market area, Doimukh, Papum Pare, Arunachal Pradesh, Northeast India. The leaves were pounded into fine flakes, then shade-dried and kept in airtight containers. Black ginger rhizome samples were collected from a home garden (Banderdewa, Papum Pare, Arunachal Pradesh, India). The rhizomes were washed thoroughly, sliced into thin slices, and dried in a tray drier at 50 °C for 5 - 6 h before being flaked and stored for extraction and for preparing the mixed tea bags.

Preparation of black ginger flakes for green tea

Black ginger rhizomes (*K. parviflora*) were thoroughly washed to remove surface impurities, sliced into thin pieces and subjected to thermal treatment for 2 - 4 min at 45 - 50 °C and dried in a hot tray dryer at 55 - 60 °C for 5 - 6 h until fully dried. The dried slices were then ground into flakes and stored in an airtight container at room temperature, protected from light (Figure 2).

Sample preparation

The three green tea samples were prepared with different fortification levels of black ginger powder, which were coded as T2, T3, and T4, along with a control sample T1 containing only green tea. T2 contains 1 g green tea + 1 g black ginger + 1 g cardamon, T3 is made up of 2 g green tea + 1 g black ginger, and T4 contains 1 g green tea + 1.5 g black ginger + 0.5 g cardamon. Each sample was brewed by steeping 3 g of tea bag in 150 mL of hot water at 80 °C for 3 min, following

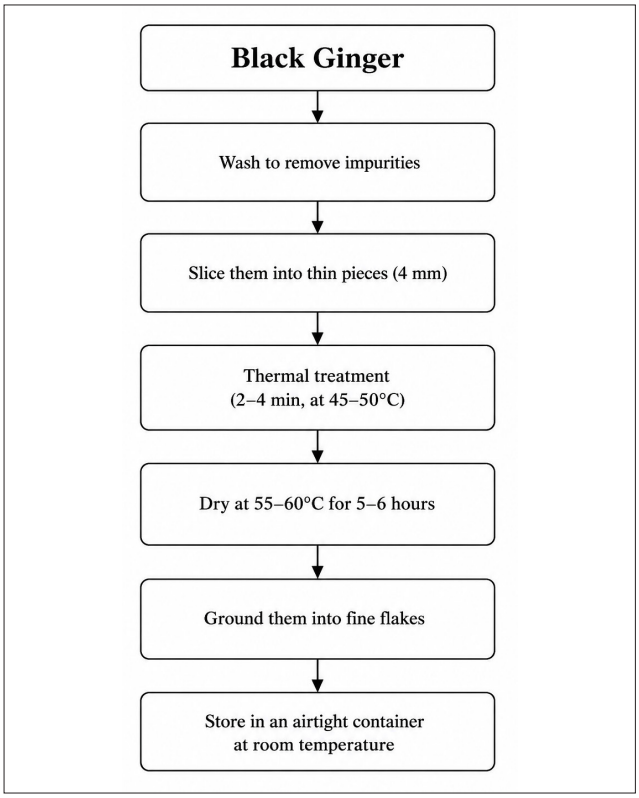

Figure 2: Flow chart for manufacturing black ginger flakes.

standard infusion protocols for sensory evaluation [14]. In some formulations, cardamom was added as a natural flavoring agent to counteract the pungency and bitterness contributed by black ginger and to enhance consumer acceptability. T3 was prepared without cardamom to assess the sensory attributes of black ginger-green tea without flavor masking. Hence, the formulations were designed to evaluate both the flavor optimization and consumer preference, not the isolated effect of black ginger concentration.

Sensory evaluation

Using the 9-point hedonic scale, a sensory evaluation was carried out to determine how consumer-acceptable green tea samples are. When developing any food products, this approach is frequently utilized to ascertain consumer preferences based on their emotional reactions [15]. Twenty trained panelists, ranging in age from 18 - 45, were chosen from among Rajiv Gandhi University's faculty and students to take the sensory test. As advised by sensory investigations, all participants provided informed consent before the examination and were frequent tea drinkers [15]. To prevent prejudice, the tea sample was presented in odorless paper cups that were randomly coded with three-digit numbers.

Data analysis

The data were analyzed using IBM SPSS Statistics version 25.0. Significant differences among treatments were determined by one-way analysis of variance. Where significant differences ($p < 0.05$) were found, means of treatments were compared using Duncan's Multiple Range Test (DMRT). Results are expressed as mean ± standard error of the mean (SEM) (or mean ± standard deviation (SD), as you actually report). Different superscript letters are significant differences among treatments at $p < 0.05$ [14]. The sample receiving the highest overall acceptance score is chosen for additional analysis. T2 was selected for further extraction, chemical and functional analysis, including TPC, DPPH assay, and proximate analysis composition.

Ash content determination

According to Wahyuni et al. [16], the dry ashing method was used to ascertain the ash concentration of green tea samples. A pre-weighed crucible was filled with about 3 g of a finely ground green tea sample, which was then burned for 6 h at 550 °C in a muffle furnace until its weight remained constant. Later, the crucibles were cooled in a desiccator before being weighed once more. The amount of ash was calculated using equation 1.

$$Ash\ content\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100 \quad (1)$$

Determination of moisture content

With minor adjustments, the gravimetric oven-drying method was used to measure the samples' moisture content in accordance with standard procedures outlined by Roshanak et al. [17]. A precisely weighed 3 g sample of finely powdered grains was put in a previously weighed and dried petri plate. After that, the samples were dried for 4 h at 105 ± 1 °C in a hot air oven or until their weight was constant. In order to determine the moisture content, equation 2 was utilized.

$$Moisture\ content\ (\%) = \frac{Before\ dry\ weight - After\ dry\ weight}{Before\ dry\ weight} \times 100 \quad (2)$$

Crude fat content determination

The Soxhlet extraction method, as outlined by the Association of Official Agricultural Chemists [18], was used to ascertain the crude fat content of the green tea fortified with black ginger. 3 g of a finely ground tea sample and an oven-dried sample were accurately weighed and placed into a fat-free extraction thimble made of filter paper. Petroleum ether, which has a boiling point of 40 - 60 °C, was used as the solvent for the extraction process after the thimble was placed inside a Soxhlet apparatus. The extraction process was continued for approximately 6 h, ensuring continuous reflux and solvent cycling over the sample. Following the completion of the extraction, the solvent was recovered by distillation, and any leftover solvent was eliminated by drying the remaining fat at 105 °C in a hot air oven. After cooling in a desiccator, the flask was weighed. The percentage of fat was found by applying equation 3.

$$Fat\ content\ (\%) = \frac{Weight\ of\ extracted\ fat}{Weight\ of\ sample} \times 100 \quad (3)$$

Crude fiber content determination

The crude fiber content of green tea reinforced with black ginger was evaluated using the Weende method, which simulates the digestive process in the gastrointestinal system by alternating between acid and alkali digestion. A finely powdered and defatted material weighing 3 g was placed in a fiberglass crucible. The sample was first digested with 1.25% sulphuric acid for 30 min at boiling temperature. Later the

residue was filtered and with thoroughly washed hot distilled water to remove any acid. Next, the sample was subjected to alkaline digestion using 1.25% sodium hydroxide (NaOH), also for 30 min under boiling conditions. The resulting mixture was filtered, washed again with hot water, and finally with ethanol and acetone to remove any remaining impurities or fat residues. The residue was dried in an oven at 105 °C for 5 h, cooled in a desiccator, and weighed. It was then incinerated in a muffle furnace at 550 °C for 4 h to obtain the ash content. In order to determine the crude fiber content, equation 4 was used.

$$Crude\ fiber\ (\%) = \frac{W_1 - W_2}{W} \times 100 \quad (4)$$

Where, W_1 is the weight of dried residue; W_2 is the weight of ash; and W is the weight of the original sample.

Aqueous extraction and yield by percentage mass

To separate the water-soluble substances from the green tea samples, aqueous extraction was used. In a beaker, a known amount of black ginger fortified green tea (3 g) was combined with 80 mL of distilled water after being weighed. To aid in extraction, the mixture was heated to 80 °C for 30 min while being constantly stirred [19]. The liquid extract was separated from the residue by cooling the mixture and filtering it through Whatman No. 1 filter paper. A hot air oven set between 50 °C and 60 °C was used to collect the filtrate and evaporate it until it was completely dry. The yield of water-soluble chemicals was measured by weighing the resultant dry extract. The extraction yield as a percentage (by mass) was computed using equation 5.

$$Extraction\ yield\ (\%) = \frac{Mass\ of\ dried\ extract}{Initial\ dry\ sample\ weight} \times 100 \quad (5)$$

Determination of titratable acidity

Using the conventional acid-base titration method, the titratable acidity of the black ginger-fortified green tea was measured to determine its overall acidity. To make a sample of the tea infusion, 1 g of the dried tea sample was steeped in 100 mL of distilled water at 95 °C for 5 min. Whatman No. 1 filter paper was then used to filter this. 10 mL of the filtrate were taken as an aliquot and mixed with 10 mL of distilled water in a conical flask. A few drops of phenolphthalein indicator were added to the diluted sample. The solution was then titrated against a standard solution of NaOH at 0.1 N. Two to three drops of phenolphthalein indicator were applied to the sample. The solution was then titrated using a standardized 0.1 N NaOH solution. The endpoint was reached when a slight pink tint developed and persisted for at least 30 s. The titratable acidity was calculated and expressed as a percentage of citric acid equivalents using equation no. 6.

$$Titratable\ acidity\ (\%) = \frac{Volume\ of\ NaOH \times Normality \times Equivalent\ weight\ of\ citric\ acid \times 100}{Volume\ of\ sample \times 1000} \quad (6)$$

The equivalent weight of citric acid used for calculation was 64.04 g/mol. The values reported are the means of triplicate data. This enables the quantification of total organic acid content contributing to the flavor profile and preservation potential of the product.

Determination of the antioxidant level of green tea fortification with black ginger

Extraction of the sample for antioxidant analysis

For extraction, 3 g of the sample (green tea and black ginger) were mixed with 50 mL of 99% methanol. The mixture was subjected to ultrasonic-assisted extraction using an ultrasonic bath at 40 °C for 15 min and kept overnight, and the mixture was filtered through Whatman No. 1 filter paper, stored in an airtight glass bottle and kept in the refrigerator.

Determination of TPC

The black ginger-fortified green tea's TPC was determined using the Folin–Ciocalteu colorimetric technique, with a few minor adjustments made to account for the sample matrix. 0.5 mL of the aqueous extract and 2.5 mL of the 10% Folin-Ciocalteu reagent were put into a test tube. Before adding 2.0 mL of a 7.5% (w/v) sodium carbonate solution, the mixture was left to react for 5 min at room temperature. Following that, the mixture was vortexed and left to stand at room temperature in the dark for 2 h. The absorbance was measured using a UV-Vis spectrophotometer at 765 nm. A standard calibration curve was generated ranging in concentration from 0 to 800 µg/mL using gallic acid standard. The TPC of the samples was determined using the gallic acid calibration curve and expressed as µg GAE/mL of the extract solution.

Determination of DPPH radical scavenging activity

Antioxidant activity of black ginger-fortified green tea was determined by the DPPH radical scavenging assay according to Paiva et al. [20]. Different doses of the green tea extract were mixed with 0.1 mM DPPH solution in methanol and was incubated for 30 min at room temperature in the dark room. Then the absorbance at 517 nm was noted using UV-Vis spectrophotometer. The percentage of DPPH radical was calculated using equation 7.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_o - A_s}{A_o} \times 100 \quad (7)$$

Where, A_s is the sample absorbance and A_o is the blank solution absorbance.

Results and Discussion

Sensory assessment

The results indicated that T2 was the preferred sample with the highest scores in taste, aroma and overall acceptability (8.0 ± 0.2, 7.8 ± 0.3, and 8.1 ± 0.2, respectively) (Table 1). The low-level addition of black ginger in equal ratio with green tea has probably boosted the flavor without any overwhelming bitterness or spiciness. The panelists described T2 as "pleasant", "mildly spicy", and "balanced" which suggests that the combination at this level was synergistic. In contrast, T4 prepared with the highest concentration of green tea and black ginger blend had the lowest rating in all aspects like aroma, appearance, taste, aftertaste, and color with an overall acceptability score of 5.4 ± 0.5. Many panelists commented that the flavor was too strong, bitter or sharp, especially in the aftertaste. This has been a common sensory limitation in herbal

Table 1: Sensory score of green tea fortified with black ginger.

Attribute	T1	T2	T3	T4
Appearance	6.8 ± 0.3	7.5 ± 0.2	7.1 ± 0.3	6.0 ± 0.4
Aroma	6.5 ± 0.4	7.8 ± 0.3	6.9 ± 0.4	5.7 ± 0.5
Taste	6.6 ± 0.5	8.0 ± 0.2	7.2 ± 0.4	5.3 ± 0.6
Aftertaste	6.4 ± 0.4	7.9 ± 0.2	7.0 ± 0.3	5.2 ± 0.5
Color	6.7 ± 0.3	7.6 ± 0.2	7.3 ± 0.3	6.5 ± 0.4
Overall acceptability	6.7 ± 0.3	8.1 ± 0.2	7.0 ± 0.3	5.4 ± 0.5

Note: Values are mean ± standard error, n = 20.

fortification as when it exceeds a certain threshold it can lead to poor consumer acceptability because of strong bioactive flavors [15, 21]. T1 (control) and T3 received moderate scores where T1 was found to be familiar in flavor and it lacked the complexity brought by black ginger. However, T3 showed has shown some improvement in comparison to T1 (6.7 ± 0.3) in terms of aroma and taste but was less preferred than T2 (7.0 ± 0.3). These findings further suggest that incorporating equal ratio of black ginger and green tea is most acceptable at low concentrations, as seen in T2. Thus, T2 can provide more functional benefits without compromising sensory quality which makes it ideal for product development.

The results show that the fortification of green tea with black ginger has a significant effect on the sensory profile of green tea and that the preferred formulation was T2 (Figure 3). The relatively high scores of T2 for all attributes and especially high scores for taste and overall acceptability indicated that the black ginger concentration or preparation method in T2 was at the right balance and did not negatively affect the green tea taste. The small standard errors for T2 also support consistency and strong positive perception among the sensory panelists. Further studies may include determining the specific concentration of black ginger for each treatment and testing consumer preference with a larger and more diverse population to support these findings. Furthermore, investigation on the specific compounds in black ginger responsible for the sensory enhancement in T2 and the negative attributes in T4 could further provide deeper insights into flavor chemistry and product optimization.

Proximate composition analysis

Ash content determination

The ash content of the black ginger fortified green tea was 9.49 ± 0.07% on a dry weight basis. The value represents the total mineral residue after complete burning of the organic matter and is an important indicator of the mineral composition of the sample. The ash content obtained in the fortified sample was slightly higher than the usual range of values reported for the green tea which usually ranges between 4.0% and 7.0% [22]. The higher ash content is associated with the inclusion of black ginger (*K. parviflora*) as it is known for its high mineral content, such as potassium, magnesium and calcium [23]. The high ash content of the tea indicates that it may have a higher nutritional value with the addition of valuable minerals, in addition to its bioactive compounds. The value is also within the permissible range of herbal and functional beverages, indicating good formulation quality and safety [24]. These results are in agreement with other studies that have reported the addition of medicinal herbs such as turmeric and ginger to tea blends increased the amount of ash due to the cumulative mineral contribution [25]. Moreover, the high ash content could also affect the extraction efficiency of bioactive compounds during infusion, because minerals can change the ionic environment and increase the solubility of phenolic constituents [25]. Besides, minerals like magnesium and calcium are involved in different metabolic and physiological functions which enhance the functional utility of the fortified drink. Similar findings have been noted in herbal formulations where fortification with plant-based ingredients improved mineral profiles without compromising the safety or quality of the product [26]. Therefore, the outcome indicates the benefit of using black ginger for functional fortification in green tea in the development of nutritionally enriched beverages.

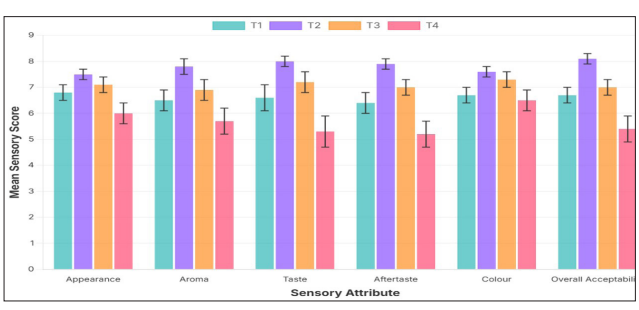

Figure 3: Mean sensory score graph.

Moisture content determination

The moisture content of the green tea fortified with black ginger was found to be 1.289 ± 0.05% which reflects a high degree of dryness and ideal storage conditions for a botanical product in powder form. This can be attributed to efficient drying processes that minimize the amount of water left behind, reducing microbiological proliferation, enzymatic activity and chemical breakdown whilst in storage. The moisture value was much lower than the previously reported values for green tea-based goods. As an example, Xu et al. [27] reported the moisture content of commercially available green teas to be in the range of 2.50 - 4.75%. Likewise, Das and Eun [28] reported moisture of 3.12% in green tea granules for antioxidant rich infusion. These values are higher than the current formulation indicating the possibility that the green tea fortified with black ginger might have improved shelf stability and lower risk of quality deterioration. The low moisture content observed may further support the conservation of phenolic compounds and antioxidants, as green tea and black

ginger are known to be rich in polyphenols. Moreover, excess moisture can lead to hydrolysis and oxidation, reducing the product's efficacy as a functional beverage [29]. Therefore, retaining a moisture level below 2% is likely to retain higher antioxidant integrity. Furthermore, the low moisture content also contributes to better storage stability by preventing clumping in powdered formulations and ensuring improved solubility during infusion. Similarly, according to standard specifications the moisture content should be below 5% in dried tea products to ensure quality and prevent spoilage (ISO 3729:2011). The low moisture content also helps in minimizing the potential need for artificial preservatives while making the product more appealing to health-conscious consumers.

Crude fat content determination

The fat content of the black ginger fortified green tea was found to be 0.62 ± 0.01% on a dry weight basis. This finding supports both green tea leaves and black ginger rhizomes as low-fat botanically identified for their low lipid concentrations. The low-fat value also aligns with the nutritional expectations of green tea products making it suitable for health-conscious individuals looking for low-calorie and low-fat beverage options. Similar studies on green tea and herbal tea products also confirm this value, where study by Xu et al. [27] found that the fat content of green tea samples varied from 0.60 to 1.20 per cent depending on drying techniques and leaf maturity. Similarly, Saeed et al. [30] has discovered that green tea incorporated with turmeric and ginger had fat content ranging from 0.65 to 0.92%. Furthermore, proximate analysis of green tea yielded similar results where fat content values varied from 0.50% to 1.10% in different cultivars and processing conditions, thus further confirming that lipids comprise only a minor fraction of tea leaves [22]. Moreover, studies on herbal tea formulations enriched with plant extracts have also reported fat content of 0.55% to 0.95%, indicating less significant effect of incorporated bioactive ingredients in lipid profile of the final product [31]. Therefore, present finding of 0.62 ± 0.01% is consistent with the reported ranges and further supports the stability and nutritional suitability of the formulation. Low fat content is nutritionally desirable as it minimizes the risk of lipid oxidation, increases shelf-life and maintains the product within a desirable calorie range.

However, despite having low fat content, they may still include small quantities of beneficial unsaturated fatty acids that are naturally present in tea leaves and rhizomes [30]. These trace amounts of fat can help in the absorption of fat-soluble phytochemicals such as flavonoids, enhancing the overall bioavailability of antioxidants in the beverage. The low-fat functional beverages are also aligned with dietary goals of consumers aiming at reducing saturated fat intake and managing weight. The addition of black ginger did not significantly elevate the fat content, indicating that fortification enhanced phytochemical properties without affecting the nutritional leanness of the product.

Crude fiber content determination

The crude fiber content was found to be 19 ± 0.54% which is relatively high value, indicating the rich dietary fiber content in product. The fibrous nature of black ginger rhizomes and green tea leaves are both known for their plant cell wall constituents like cellulose, hemicellulose, and lignin which is the cause of increased crude fiber content [32, 33]. The addition of black ginger may have a significant role in the increased fiber content as rhizomes naturally contain more structural fiber than tender leaves.

According to the previous study by Demir et al. [31] on green tea, the crude fiber content of 19 ± 0.54% exceeds the typical range reported for green tea products. For instance, study done by Zhao et al. [32] has recorded around 11.2% of crude fiber value for dried green tea leaves whereas Gosavi and Swami [33] have reported 13.5% of crude fiber content in green tea infused with ginger extract. The high crude fiber content obtained in this study showed that the addition of black ginger has significantly improved the dietary fiber level of the product. This finding is nutritionally relevant because a product with 19 ± 0.54% crude fiber content can serve as a functional beverage that can contribute to daily fiber intake, especially for consumers seeking plant-based high fiber alternatives. Additionally, similar studies on herbal and functional tea formulations have shown crude fiber values range from 10.0% to 16.8% depending on the type of plant material and level of fortification [26]. Thus, the higher value found in the present study may further highlight the contribution of black ginger as a fiber-rich ingredient. Moreover, dietary fiber from plant sources has also shown to enhance satiety and support metabolic health which further reinforce the functional significance of the developed product. These findings suggest that the formulated tea exceeds conventional fiber contents reported in other green tea beverages, thereby offering added nutritional benefits. Crude fiber has also shown better glycemic management, lower risk of cardiovascular diseases, and development of healthy gut flora [32]. Additionally, high fiber content supports claims related to gut health and wellness which potentially increase consumer appeal. However, it can also influence the solubility and texture of the beverage during the preparation which should be considered in formulation and marketing.

Aqueous extraction yield

The aqueous extraction yield of the green tea fortified with black ginger was found to be 21.34 ± 0.3%. This represents the percentage of soluble solids extracted from the tea blend into water under defined brewing conditions and provides an indication of the product's ability to release its bioactive and nutritional constituents. The obtained yield was found to be within the standard range between 15% to 30% for herbal infused tea, depending on the botanical composition, particle size and extraction conditions [30]. The addition of black ginger, which contains water-soluble flavonoids and phenolic compounds has likely contributed to the observed extractability. The higher concentrations of soluble bioactive substances like flavonoids, polyphenols and antioxidants have found to be associated with higher aqueous extraction yields [32]. Similar findings were found in results herbal blends containing rhizomes and leaves exhibiting enhanced extractive values due to synergistic solubility of their phytoconstituents [24]. However, the efficient extraction also indicates better solubility and potential consumer acceptability in terms of

flavor and functionality. Additionally, study by Demir et al. [31] on Turkish green tea and herbal infusions has also reported aqueous extraction yields in the range of 18.5% to 26.7%, depending on extraction temperature, time and particle size of the plant material. The value obtained falls well within this range which further confirms the efficiency of the extraction process. Furthermore, higher extraction yield has also positively linked to increased TPC and antioxidant activity, indicating improved functional quality of the beverage [25]. The results suggest that, in addition to improving the phytochemical composition, the addition of black ginger promotes the effective release of these compounds during the infusion process, thus improving the overall functional performance of the developed tea.

Titratable acidity determination

The titratable acidity of the green tea fortified with black ginger was 0.263 ± 0.01% expressed as citric acid equivalents. This value represents the number of organic acids present in the drink which are responsible for the taste, pH stability and the preservation of the formulation. The level of acidity observed is within the normal range for brewed herbal teas and functional beverages which is generally 0.20% to 0.35% depending on the botanical ingredients used [34]. The low acidity may be a result of the natural organic acids of green tea (gallic and quinic acid) and the phenolic components of black ginger which can donate protons during titration, thereby contributing to the overall titratable acidity. A titratable acidity of 0.263 ± 0.01% supports a balanced flavor profile without excessive sourness which may be favorable from a sensory perspective. This acidity level may also enhance the overall mouthfeel and taste perception while complementing the bitterness and astringency of tea catechins. Additionally, acidic conditions are also known to stabilize phenolic compounds and delay microbial spoilage which potentially improve the shelf life of the fortified beverage [25]. The value further aligns with the findings from similar studies done on green tea products combined with herbal extracts such as ginger, lemon or hibiscus which have shown titratable acidity value ranging from 0.22% to 0.30% [35]. Another study on herbal tea infusions has reported similar titratable acidity values ranging from 0.24% to 0.32% depending on the type of plant material and infusion conditions [31]. Similarly, evaluation of composite herbal teas blends by Caleja et al. [24] has also demonstrated acidity values around 0.27%, indicating that incorporation of plant-based bioactive does not significantly disrupt the acid balance of the beverage. These findings further confirmed that the observed acidity was within the optimal range in maintaining both chemical stability and sensory acceptability of the developed formulation.

Determination of antioxidant potential

TPC determination

The TPC was measured against a gallic acid standard curve using a linear regression equation (Figure 4). The incorporation of black ginger extract into green tea has shown significant increase in phenolic content by indicating a clear upward trend observed as the extract concentration increased. For extract concentrations of 10, 20, and 30 µg/mL, the corresponding phenolic content values, as determined by the gallic acid standard curve, were 506.0 ± 1.7, 553.0 ± 1.6, and 573.3 ± 1.6 µg GAE/mL (Table 2). This pattern demonstrated a dose-dependent enrichment of phenolic compounds which reflect the bioactive potential of black ginger.

Moreover, there were significant differences in TPC values among tested concentrations ($p < 0.01$), which means that the variance is not caused by random error, but rather due to the increased concentration of phenolics from black ginger. These results are in line with previous studies indicating that black ginger is rich in flavonoids and methoxy flavones, which are known to have antioxidant activity [34]. These substances are known to react with the Folin–Ciocalteu reagent and are efficient reducing agents, thus increasing the absorbance readings of the assay.

In addition, green tea is itself high in catechins such as EGCG, which are powerful antioxidants. The addition of black ginger would probably improve the antioxidant profile of the beverage due to the synergistic effect. Previous studies have shown that the combination of botanical extracts can produce additive or even synergistic effects on TPC and antioxidant activity [36]. In this case, the TPC from green tea and black ginger reinforces the functional value of the final product. The smaller difference in TPC between 20 and 30 µg/mL indicates a possible plateau in phenolic absorption or reaction under the conditions of the assay. This observation further implies the increasing black ginger concentration can enhance phenolic content whereas there may be an upper limit to measurable or

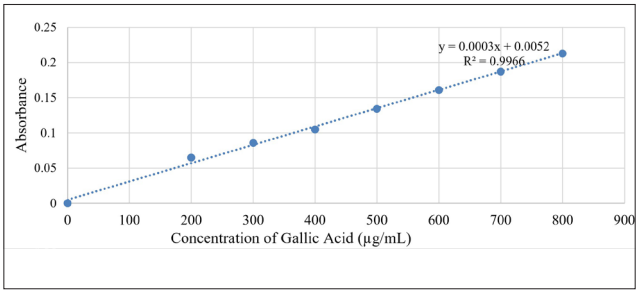

Figure 4: Standard calibration curve of gallic acid showing the relationship between absorbance and concentration (µg/mL).

Table 2: Absorbance values obtained during the TPC assay of green tea fortified with black ginger at different extract concentrations.

Extract (µg/mL) conc.	Abs. 1	Abs. 2	Abs.3	Mean	SD	SEM	P value
10	0.129	0.163	0.179	0.157 ± 0.03[a]	0.0255	0.01474	<0.01
20	0.146	0.178	0.189	0.171 ± 0.01[b]	0.0223	0.01289	<0.01
30	0.151	0.181	0.199	0.177 ± 0.01[b]	0.0242	0.014	<0.01

Note: SEM = SD/√n; values are expressed as mean ± SEM (n = 3). Means with different superscript letters within the same column differ significantly according to DMRT at $p < 0.05$.

bioavailable phenolics in the system.

The TPC in green tea-based and herbal formulations ranged from 450 to 620 µg GAE/mL in other studies which closely matches the present findings [25]. For example, the evaluation of herbal extracts showed TPC values of around 500 - 580 µg GAE/mL, which reveals that the addition of bioactive-rich plant materials significantly increases phenolic concentration. Moreover, a strong correlation between the increased TPC and antioxidant activity, especially in DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays, has been observed, which further supports the functional implications of the observed trend [26]. These results further confirm the increased phenolic density and potential health benefits of the supplemented tea.

DPPH determination

The DPPH radical scavenging assay was used to evaluate antioxidant activity of the green tea fortified with black ginger. Measurements were taken at one-minute intervals over four minutes, yielding the following scavenging percentages: 84.59% (1 min), 85.59 ± 0.7% (2 min), 87.13 ± 0.7% (3 min), and 88.70 ± 0.6% (4 min) (Figure 5).

This time dependent increase in DPPH radical scavenging suggests a rapid and sustained response of antioxidant activity. The maximum activity was 88.70 ± 0.6% in the 4th min which showed the strong ability of samples to donate hydrogen atoms or electrons to neutralize free radicals. The radical scavenging capacity increased progressively with time, indicating the effective and continuous interaction between DPPH radicals and the antioxidant compounds present in the extract. The increasing antioxidant activity observed is probably due to the combination of methoxy flavones in black ginger and catechins in green tea. EGCG, which is abundant in green tea, is well known for its powerful ability to scavenge free radicals [37]. Black ginger contains PMFs such as 5,7-dimethoxyflavone, which provide further antioxidant properties [12].

The scavenging activity trend indicates that the two plant ingredients could act in coincidence whereas the progressive increase with time might suggest a layered or delayed release of some antioxidant chemicals due to the numerous phytochemical interactions. Several research have shown scavenging activities of black ginger to be in the range of 60 to 80% depending on concentration and extraction methods and scavenging activities of green tea was found to be in the range of 70 to 85% [38, 39]. Hence, the observed value of 88.70 ± 0.6% after 4 min indicates an increase in the antioxidant capacity in the fortified blend.

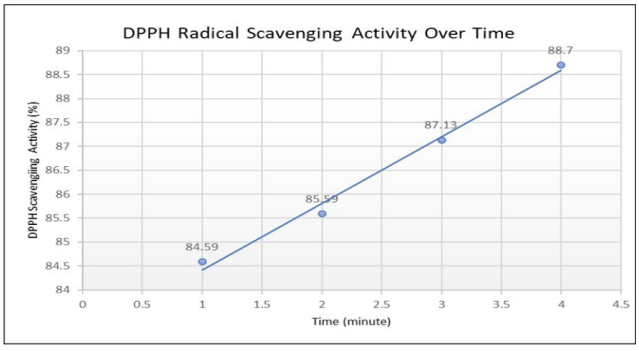

Figure 5: DPPH scavenging activity (%) over time.

Additionally, similar studies on herbal tea have also reported DPPH radical scavenging activities ranging from 75% to 90% aligning with the present findings, depending on the type of plant extract and extraction conditions [25]. For instance, study on green tea-based functional beverages enriched with plant extracts have shown around 85 - 89% of antioxidant activities, indicating improved free radical scavenging due to synergistic interactions among phytochemicals. Furthermore, increase in antioxidant activity over time can be due to progressive interaction kinetics between DPPH radicals and phenolic compounds which may further support the relationship between higher TPC and enhanced antioxidant capacity. Thus, these findings strengthen the functional potential of the developed formulation as a potent antioxidant beverage.

Conclusion

The objective of the study was to combine the two potent ingredients, such as green tea and black ginger, in a single drink that would not only meet the consumer's taste preferences but also provide health benefits. Development process includes extraction, formulation, physicochemical analysis, antioxidant capacity and sensory evaluation. The samples were prepared by aqueous extraction where water was chosen to simulate usual consumer preparation while achieving a satisfactory extraction yield of 21.34 ± 0.3%. The TPC has been found to be continuously increasing with increased concentrations, indicating the presence of compounds high in antioxidants. High radical scavenging activity was confirmed by the DPPH assay further indicating its antioxidant potential. The proximate analysis also indicated favorable characteristics for product stability and nutritional quality including low moisture, low fat, appreciable mineral and dietary fiber content which may contribute to the overall nutritional value of the developed functional beverage. In conclusion, this research successfully achieved its aim as the combination has shown to provide an appealing alternative to conventional teas, enriched with natural antioxidants and dietary fiber. This formulation has also shown promising potential for commercial production in the functional beverage market by offering a healthful and natural option backed by scientific analysis.

Future Prospects

Based on the results of this study, several recommendations and future directions are proposed to improve and expand the development of black ginger-fortified green tea as a functional beverage. Extraction was made with water to reflect present consumer preparation and manufacturing processes. In the future, the efficiency of extracting bioactive compounds such as PMFs and catechins may be enhanced by studying ethanol, methanol or hydroethanolic solvents under controlled laboratory conditions. Methanol is recommended for research and analytical applications only, to aid in the extraction and characterization of phytochemicals and not for the preparation or commercial production of food or beverage products.

Product development and commercial applications would be better served with food grade solvents such as ethanol. These solvents could also enhance the recovery of the antioxidant compounds and the TPC. Accelerated and real-time shelf-life experiments are suggested to determine long-term physical, chemical and microbiological stability of the product. The variations of antioxidant activity, phenolic content, flavor and color can be assessed to ensure long-term quality for market provision. Additionally, an in-depth economic feasibility study can also be performed to assess the cost of production, availability of raw materials, packaging and potential pricing before market introduction of product. Pilot-scale trials can further help in optimizing processing conditions for large-scale production.

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None.

Conflict of Interest

None.

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